Assessment of Larvicidal Activity of *Hyptis suaveolens* and *Balanites aegyptiaca* Leaves and Root Extracts against Mosquito Species

[1] A. A. Bobbo [2]M.S. Pukuma [3]M.A.Qadeer

[1] Department of Animal Health College of Agriculture Jalingo, Taraba state, Nigeria.
[2] Department of Zoology, M odibbo Adama University of Technology, Yola, Adamawa State, Nigeria
[3]Department of Zoology, M odibbo Adama University of Technology, Yola, Adamawa State, Nigeria

Abstract- The Continuous use of synthetic insecticides and its toxicity problem together with the growing incidence of insect resistance has called the need for novel insecticide. Plant extracts may be alternative sources that constitute a rich source of bioactive compounds that are biodegradable and environmental friendly. Therefore, the present research is aimed at assessing the larvicidal activity of *Hyptis suaveolens* and *Balanites aegyptiaca* acetone leaf and root extracts against mosquitoes larvae. World Health Organisation protocol was adopted for the larvicidal bioassay. Twenty group of fourth instar larvae were exposed to various concentrations of 20,40,60,80 and 100ppm, mortality was observed and recorded after 24 hours. . Results are mean of three replicates. Percentage mortality was calculated using Abbott's formula, while the LC50 was determined by a log dosage probit mortality regression line and mean ± standard deviation was obtained, Significant decision was made at $p<0.05$. The result revealed that, after 24hours exposure to the extracts at 100ppm *Balanites aegyptiaca* acetone leaf extracts exhibited the highest mortality rate of 60.00 % ± 1.00 at 100ppm and LC50 was achieved at 1.83±0.11 than the *Hyptis suaveolens*,leaves with only 53.33±1.16 and LC50 was achieved at 1.78 ± 0.97ppm, while *B. aegyptiaca* root possess the highest mortality rate of 63.33±0.58 compared to *H. suaveolens* with 61.67±1.53% mortality . Findings also revealed that mortality was concentration dependent and there were significant difference between dependent and responses at $p<0.05$. Root extracts also showed better mortality rate compared to the leaves extracts. Both the experimental plants contained some potent of larvicidal activity which might be suitable alternative to chemical larvicide. It is recommended further research should be carried out on the larvicidal activity at a higher time of 48 and 72hrs to determine its potency.

Index Terms- *Hyptis suaveolens Balanites aegyptiaca,* L arvi ci dal properties, L C50.

I. INTRODUCTION

Mosquitoes are the oldest human enemy and represent a significant threat to human health because of their ability to vector pathogens that cause diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encepahalitis and Filariasis that afflict millions of people worldwide (Service, 1983; Gubler, 1998; WHO 1992). The continuous use of synthetic insecticides, its toxicity problem together with the growing incidence of insect resistance has called the need for novel insecticide. Plant extracts may be alternative sources that constitute a rich source of bioactive compounds that are biodegradable and environmentaly friendly.

Hyptis suaveolens belongs to the family *laminaeceae* and is a native tropical America, but it is wide spread in tropical Africa. Asia and Australia, it grows under a wide variety of soil and climate, mainly in warm area (Peerzada, 1997). *Hyptis suaveolens* (L.Poit) commonly called bush mint, bush tea, pignut, is known as vilayati tulsi in hindi; konda thulasi in Telugu; bhustrena in Sanskrit; daddoya-ta-daji in Hausa; *efiri* in Yoruba; nchuanwu in Ibo; tanmotswangi-eba in Nupe and kachu-kachugha in Fulfulde. *Hyptis suaveolens* is a very common plant found along roadsides and farms in different parts of the world mainly in the tropics and subtropics. *Hyptis suaveolens* has both medicinal individuality as well as insecticidal properties and it's used for traditional medicine for treatment of various illnesses (Peerzada, l997). The leaves of the plant have shown to contain alkaloids, terpenes and volatile oil (Gills, 1992). Reported pharmacological activities of the plant include anti-inflammatory (Grassi *et al*., 2006), antiulcer (Das *et al.*, 2009), antioxidant (Gavani and Paarakh, 2008), insecticidal (Adda *et al*., 2011) and antibacterial (Asekurn *et al*., 1999). Leaves of plant have been traditionally used as a stimulant against cold and diarrhea (Beams, 1994). Fumes of the dried leaves are also used to repel mosquitoes and control insect's pest of stored grains. Leaves are used in the preparation of mint flavoured beverages. Roots are chewed with betel nuts as a stomaclic and its decoction is used as an appetizer **(**Ambasta,1981**).** The ethanolic extract of *Hyptis suaveolens* was examined for its toxicity effect on the larvae of the yellow fever mosquito *Aedes aegypti* **(**Bhagwat and Umathe, 2003**).** Extracts of various parts of *Hyptis suaveolens* have been obtained with solvents like petroleum ether, chloroform, methanol, ethanol, n-hexane, and water using soxhlet extraction, cold maceration, and steam distillation methods (Edeoga *et al.*, 2006; Prasanna, 2012; Sulta, 2013) and subjected to phytochemical screening using standard methods (Harbourn, 1973; Hang, 1983).

Desert date (*Balanites aegyptiaca* (L) Del.), of the family *Balanitaceae*, is indigenous to all dry land south of Sahara and extending southwards (Hall and Walker, 1991). It is an evergreen tree adapted to various climatic conditions especially in arid

regions with extremely high temperature and scarce water, thus helps in combating desertification (Gour and Kant, 2012). The tree is widely distributed in many tropical countries of Africa and Asia . The flowering time generally occurs during November-April, while the fruiting takes place during December-July. The tree is rich is medicinal ingredients and contains other useful products with multi use in rural lives and industry. Among such useful products, high level of oil (30-60%) can be extracted from seeds with valuable application as cooking oil as well as biofuel (Moktar and Abdallah, 2013). Secondary metabolites like rotenone, bergopin, steroids and flavonoids were detected in different parts of the tree. Also, *B. aegyptiaca* is named as an African-Asia saponins producing plant due to its high constituent of saponin compounds. These multiple chemicals have proved different biological activities including moluscicidal, larvicidal, mosquitocidal and insect antifeedant properties, beside other industrial uses (Moktar and Abdallah, 2013). Gajalakshim *et al.* (2003) reported that *B. aegyptiaca* contain active component, saponin that prove to have insecticidal properties against *Tribolium castaneum.* Chothani and Vaghasiya (2011) also asserted that fruits kernel of *B. aegyptiaca* show larvicidal properties against *Anophelis arabiense* and *Aedes aegypti*.

II. METHODOLOGY

2.1 Collection of plant materials

Two different plants were used for the study i.e *Hyptis suaveolens* (bush tea) and *Balanites aegyptiaca* (Balanitaceae). The plant parts was collected in and around Jalingo metropolis, and was authenticated by a Taxonomist from the Department of Plant Science MAUTECH.

2.2 Collection and Rearing of larvae

Larvae of mosquitoes were collected from any available stagnant water within and around Jalingo metropolis using dipper and pipette as described (Adeleke *et al*., 2008) and was transported to the laboratory of the Modiddo Adama University of Technology Yola. Larvae were reared in a rearing tray containing tap water and cover by fine nylon mesh. The larva were feed with food containing mixture of cabin biscuit and yeast until the 4th instar larvae was reached.

2.3 Preparation of stock solution and test Concentration

The leaves and roots of the plant were shaded dried, pulverized and sieved to get a fine powder from which the extract was prepared. Methanol and Acetone extract of the plant were obtained by taking 200g of the powder leaves and root in a separate container and 200ml of the solvent was added. The cap vial was screwed and shaken vigorously to dissolved or dispersed the material in the solvent. The mixture was then be filtered through Whatmam filter paper and the filtrate was then evaporated under reduced temperature at 50°c on a water bath dryness to obtain the crude extract. The stock solution was diluted separately (WHO, 2005).

2.4 Larvicidal Bioassay

Larvicidal Bioassay was done according to a standard procedure provided by World Health Organization; Guideline for laboratory and field testing of mosquito larvicide (WHO, 2005). With slight modifications. 20 fourth instar larvae were transferred by means of strainer or droppers to small disposable test cups or vessels each containing 200m1 of water. The depth of the water in the cups was maintained between 5cm and 10cm. 0.2m1 of the stock was then added to 200m1 in the cups to obtain the desire target dosage stating with the lowest concentration of 20ppm, 40ppm, 60ppm, 80ppm and 100ppm respectively. Three replicates for each concentration and equal number of control were simultaneously set up with tap water, to which 1 ml of the solvent added. Larval food was then added to each test cup. After 24hr exposure, larval mortality was then recorded. Moribund larvae were counted as dead larvae for calculating percentage mortality. The result was then recorded on the data recording forms. If the control mortality is between 5% and 20%, the mortalities of the treated groups was corrected according to Abbott's formula (Abbort, 1925).

2.5 Precautionary Measures to Prevent larval Mortality

A broad surfaced rearing container was used with intermittent food supply, food was provided in a finely crushed and suspended form, while Scum formation was reduced by using a glass rod to regularly stir the water so as to enhance aeration.

2.6 DATA ANALYSIS

LC50 value was calculated from a log dosage, probit mortality regression line at 95% CL of upper confidence limit (UCL) and lower confidence level (LCL). Using Abbot's formula percentage mortality was calculated and the mean ± standard deviation presented. Analysis of variance (ANOVA) was used to determine the significant differences between treatments. Regression analysis was used to determine the relationship between concentrations and mortality rate. Results with $p<0.05$ will be considered statistical significance.

III. RESULTS

3.1 Larvicidal Activity of Acetone Leaf Extracts on Fourth Instar Larvae of Mosquito.

Table 1 shows the percentage mortality of the 4th instar larvae of mosquito exposed for 24hrs to various concentrations of acetone extracts of the two plants. The larvicidal activity of *H. suaveolenss* leaves showed, 30.00%, 31.67%, 41.33%, 43.33% and 53.33% mortality when exposed to various concentrations of 20,40,60,80 and 100ppm respectively. The *H.suaveolens* achieved the highest mortality of 53.33% at 100ppm and LC50 at 1.16 ppm, while the lowest mortality rate of 30.0% at 20 ppm and LC50 achieved at 1.78ppm. The *B. aegyptiaca* leaves recorded a result of 40.00%, 40.00%, 45.00%, 48.00% and 60.00% for the same concentrations of 20,40,60,80 and 100 respectively. Lowest mortality of 40.00% was recorded in 20ppm and the highest mortality of 60% in 100 ppm

3.2 Larvicidal Activities of Acetone Root Extract on Fourth Instar Larvae of Mosquito

Table 2 showed the result of percentage mortality of acetone extracts of the two experimental plants parts on mosquito larvae. *H. suaveolens* showed percentage mortalities to be 23.00%, 31.00%, 38.33%, 51.67% and 61.67% for concentrations of 20,40,60,80 and 100ppm. while *B. aegyptiaca* root showed 26.67%, 30.00%, 40.00%, 58.33%, 63.33% . Highest mortality of 63.33% was obtained in *B. aegyptiaca* root and the lowest percentage of 26.67% was recorded.

3.3 LC50 with (95%) limits of the tested plant extracts against mosquito species.

Table 3: Shows the lethal concentration LC50 with fiducial limit of the tested plant extract against mosquito species the LC50 value of 1.78ppm with LCL of 4.11 and UCL of 5.18 were recorded for *H. suaveolens* leaves. In the same way, *B. aegyptiaca* leaves achieved LC50 at 1.83 ppm with LCL of 4.42 and UCL of 5.25, while *H.suaveolens root* shows 1.79ppm with LCL of 4.12 and UCL of 5.20., *B. aegyptiaca* root showed an LC50 at 1.75 with LCL of 4.10 and UCL of 5.11.

Table 1 Percentage Mortality of twenty Mosquito Larvae (20) Exposed for 24 hours to Different Concentrations of Acetone Leaf Extracts in Parts Per Million (ppm)

	Observed Percentage Mortality of Larvae	
Concentration in ppm	*H, suaveolens*	*B. aegyptiaca*
control	0.00 ± 0.00[a]	0.00 ± 0.00[a]
20	30.00 ± 1.00[b]	40.00 ± 1.53[a]
40	31.67 ± 0.58[b]	40.00 ± 153[a]
60	41.67 ± 1.73[b]	45.00 ± 1.58[a]
80	43.33 ± 1.53[a]	48.00 ± 1.16[a]
100	53.33 ± 1.16[b]	60.00 ± 1.00[a]

Values with same superscript on the same column are not significantly different from each other p<0.05. Values are ± ±SD of 3 replicates.

Table 2 Percentage Mortality of twenty Mosquito Larvae (20) Exposed for 24 hours to Different Concentrations of Acetone Leaf Extracts in Parts Per Million (ppm)

	Observed Percentage Mortality of Larvae	
Concentration in ppm	*H. suaveolens*	*B. aegyptiaca*
Control	0.00 ± 0.00[a]	0.00 ± 0.00 a
20	23.00 ± 0.58[b]	26.67 ± 0.58[a]
40	31.00 ± 1.00[a]	30.00 ± 0.09[a]
60	38.33 ± 1.53[b]	40.00 ± 1.00[a]
80	51.67 ± 1.53[a]	58.33 ± 0.58[a]
100	61.67 ± 1.53[a]	63.33 ± 0.58[a]

Table 3 LC50 with (95%) limits 0f the tested plant extracts against mosquito species.

Plant Extract	LC50(ppm)	LCL(ppm) LCL (ppm)	ULC(ppm)
H.suaveolens leaves	1.78	4.11	5.18
B. aegyptiaca leaves	1.83	4.42	5.25
H. suaveolens root	1.79	4.12	5.20
B. aegyptiaca root	1.75	4.10	5.11

LC50=

Lethal concentration of 50% Mortality. LCL= lower confidence limit, ULC= upper confidence limit, at p< 0.05.

4.1 DISCUSSION

Mosquitoes in the Larval stage are attractive targets for pesticides because they breed in water sources. However, introduces many risks to people and or the environment and due to the continuous increase in their resistance to familiar synthetic insecticide, better alternative means are sought (Hag *et al.,*1999). Natural pesticides, especially those derived from plants are more promising in this aspect (Ameer and Mehlhorn, 2006). A considerable plant derivative has shown to be effective against Mosquitoes Larvae with a safer manner. In this study acetone leaves and root extracts of *Hyptis suaveolens* and *Balanites aegyptiaca* were found to have some Larvicidal activity against Mosquito Larvae at various Concentration rate in parts per million (ppm) as shown in table 1-2. The larvicidal activity was found to be higher against the fourth instar larvae at the highest concentration rate of 100 ppm. This is in accordance with the result (Rawani *et al.,* 2009), that the higher mortality rate was recorded with increase in Concentration against Mosquito species. The result revealed that maximum larvicidal activity was observed in *B. aegyptiaca* acetone leaf extract at 100ppm, and LC50 was achieved at 1.83 ± 0.11ppm, while the Lowest mortality rate was observed in *H. suaveolens* acetone leaf extacts and LC50 was achieved at a concentration of 1.78 ± 0.96 ppm, this results showed that *B. aegyptiaca* was more active as a larvicide compared to *H.suaveolens* which is in accord with the results (Bishnu and Zee, 2005). Also *B. aegyptiaca* acetone root extracts exhibited the highest mortality rate of 63.33 ± 0.58% at 100 ppm and LC50 was achieved at 1.75 ± 0.25 ppm, while *H.suaveolens* acetone root had the lowest mortality rate of 61.67 ± 1.53% with LC50 at 1.75 ± 0.19. The results showed that, *B.aegyptiaca* acetone root exhibited the highest mortality. These findings is in accord with the findings (Bishau and Zeev, 2005), that *B. aegyptiaca* root extracts seemed the most lethal, among the various part tested against the control of mosquito larvae. Earlier studies have shown that *Balanites* plant contain high amounts of saponins (Liu and Nakanishi, 1982; Kamel *et al.,* 1991). However, this is in contrary with the findings (Kavendan *et al.,* 2014), who reported that *H. suaveolens* demonstrated the highest mortality against Mosquito larvae. This variation might be attributed to the method of extractions as well as solvent used in the extraction process. The results obtained are not significantly different from each other at P>0.05. Both the leaves and the root of the experimental plants parts revealed some larvicidal activity at different concentration, however, *B. aegyptiaca* root has more potent in larvicidal activity compared to *H. suaveolens* root which is in agreement with the work (Olofsdofter *et al*., 2002; Zhang and Fu, 2010; Pukclai *et al.*, 2010), who reported that the root extract exudes higher amount of the bioactive compounds than the leaves and fruits, but contrary with the findings (Yadav and Panghal, 2010). that, the fruit extracts has high amount of bioactive. From the results obtained all the tested plants possessed different range of larvicidal activities which might be used as a traditional mosquito control agent. On the basis of the present investigation results acetone root extracts of *B. aegyptiaca* possessed the highest larvicidal bioactive which might be suitable alternative to chemical synthetic larvicide as they are safe, environmentally friendly, inexpensive and available everywhere in the world. It is therefore, recommended further research should be carried out on the larvicidal activity at a higher time of 48 and 72hrs so as to determine its potency.

ACKNOWLEDGMENTS

Authors are thankful to Mr. B. Bristone of the Department of plant science MAUTECH, for his assistance in identifying the plants species, also Mallam Murtala Lawan Raji principal Technologist, Biochemistry Department, Modibbo Adama University of Technology, Yola. Adamawa State, Nigeria and Mallam Ibrahim Hayatu of MAUTECH in the larvicidal bioassay is warmly acknowledged

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AUTHORS

First Author Ahmadu Alhaji Bobbo., Master's degree in view at Modibbo Adama University of Technology,Yola, Adamawa State. Special interest in Applied Parasitology and Entomology, worked on Assessment of larvicidal activity of *Hyptis suaveolens* and *Balanites aegyptiaca* extracts against mosquito species A lecturer, at College of Agriculture Jalingo, Taraba State, Nigeria Email- bobbo_ahmed@yahoo.com
Second Author –Dr.. M .S. Pukuma. PhD in Applied Entomology (senior lecturer, Zoology Dept)
Third Author Dr. M. A Qadeer PhD in Applied Parasitology, (senior lecturer, Zoology Dept)